



Glaucoma Risk Alleles in the Ocular Hypertension Treatment Study

Todd E. Scheetz, PhD,^{1,2} Ben Faga, BS,^{1,2} Lizette Ortega, BS,³ Ben R. Roos, BS,^{1,2} Mae O. Gordon, PhD,⁴ Michael A. Kass, MD,⁴ Kai Wang, PhD,^{2,3} John H. Fingert, MD, PhD^{1,2}

Purpose: Primary open-angle glaucoma (POAG) is a major cause of blindness and visual disability. Several genetic risk factors for POAG and optic nerve features have been identified. We measured the relative risk for glaucoma that these factors contribute to participants in the Ocular Hypertension Treatment Study (OHTS).

Design: Comparative series.

Participants: One thousand fifty-seven of 1636 participants (65%) of the OHTS were enrolled in this genetics ancillary study.

Methods: Samples of DNA were available from 1057 OHTS participants. Of these, 209 developed POAG (cases) and 848 did not develop glaucoma (controls) between 1994 and 2009. The frequencies of 13 risk alleles previously associated with POAG or with optic disc features in other cohorts were compared between POAG cases and controls in the OHTS cohort using analyses of variance. The 2 largest subgroups, non-Hispanic whites ($n = 752$; 70.7%) and blacks ($n = 249$, 23.7%), also were analyzed separately. The probability of glaucoma developing over the course of the OHTS was compared between participants stratified for transmembrane and coiled-coil domains 1 (*TMCO1*) risk alleles using Kaplan-Meier and Cox proportional hazards analyses.

Main Outcome Measures: Association of POAG with known genetic factors.

Results: No association was detected between the known POAG risk alleles when the OHTS cohort was examined as a whole. However, in the subgroup of non-Hispanic whites, allele frequencies at the *TMCO1* locus were statistically different between cases and controls ($P = 0.00028$). By 13 years, non-Hispanic white participants with *TMCO1* risk alleles had a 12% higher cumulative frequency of glaucoma developing than participants with no *TMCO1* risk alleles. Moreover, the Cox proportional hazard analysis demonstrated that *TMCO1* alleles increased relative risk comparable with that of some previously analyzed clinical measures (i.e., intraocular pressure).

Conclusions: The size of the OHTS cohort and its composition of 2 large racial subgroups may limit its power to detect some glaucoma risk factors. However, *TMCO1* genotype was found to increase the risk of glaucoma developing among non-Hispanic whites, the largest racial subgroup in the OHTS cohort, at a magnitude similar to clinical predictors of disease that long have been associated with glaucoma. *Ophthalmology* 2016;123:2527-2536 © 2016 by the American Academy of Ophthalmology



Supplemental material is available at www.aaojournal.org.

Primary open-angle glaucoma (POAG) is a common cause of blindness and visual disability¹⁻³ that is characterized by retinal ganglion cell death that produces a distinctive pattern of optic nerve head damage (cupping) and visual field loss. Four classic risk factors for glaucoma include increasing age, family history of disease, race, and elevated intraocular pressure (IOP).⁴

Heredity is important in the pathogenesis of POAG; however, the genetic basis of glaucoma is complex.⁵ Some cases of POAG are caused primarily by mutations in a single gene, such as myocilin (*MYOC*),⁶ optineurin (*OPTN*),⁷ or TANK binding kinase 1 (*TBK1*).⁸ Mutations in these glaucoma-causing genes are inherited in an autosomal dominant pattern and have high penetrance. Glaucoma develops in the vast majority of individuals with mutations in these genes, glaucoma develops. Consequently, such

mutations are observed only rarely in healthy individuals (i.e., those not known to have glaucoma). Mutations in *MYOC* cause POAG that is characterized by high IOP,⁹ whereas mutations in *OPTN* or *TBK1* are associated with POAG that typically occurs at low IOP.^{7,8} Together, these genes are responsible for approximately 5% of POAG cases.⁵

Other cases of POAG are caused by the combined actions of many genes and environmental factors. Genome-wide association studies (GWAS) of glaucoma patients have begun to identify these genetic risk factors. The first glaucoma risk factor, *CAVI/CAV2*, was detected by a large GWAS of POAG patients from Iceland.¹⁰ Additional POAG risk factors (*CDKN2B-AS1* and *TMCO1*) were detected by a GWAS that included especially severe cases of POAG.¹¹ Other GWAS confirmed that *CAVI/CAV2*,¹²

CDKN2B-AS1,^{13–16} and *TMCO1*¹⁷ are POAG risk alleles and identified more factors, including *ATOH7*,¹³ *SIX1/SIX6*,^{13,16} and *GAS7*.¹⁷ More recently, additional factors (*ABCA1*,^{18,19} *AFAP1*,¹⁸ *GMDS*,¹⁸ *PMM2*,¹⁹ *FNDC3B*,^{20,21} *TGFBR3*,²² *TXNRD2*,²³ *ATXN2*,²³ and *FOXC1*²³) have been detected by GWAS with larger cohort sizes.

Genome-wide association studies of normal-tension glaucoma patients have identified additional risk factors for glaucoma that occurs at IOP levels at or less than population norms, including *SRBD1*.²⁴ Another potential risk factor, *ELOVL5*, nearly met genome-wide threshold for significance²⁴ and later was studied in targeted association studies that suggested it also may be a glaucoma risk factor.²⁵ *TLR4* also has been identified as a risk factor for normal-tension glaucoma with some,^{26,27} but not all,^{28,29} association studies focused on this gene. Further GWAS of normal-tension glaucoma have detected a novel chromosome 8q22 locus¹⁵ and also have shown that a locus previously identified with studies of POAG with high IOP, *CDKN2B-AS1*, is also associated with normal-tension glaucoma.¹⁵

The genetic basis of quantitative features or endophenotypes of glaucoma also has been investigated using GWAS. Several genes that influence the magnitude of vertical cup-to-disc ratio (*CDKN2B-AS1*, *SIX1/SIX6*, *SCYL1*, *CHEK2*, *ATOH7*, and *DCLK1*) or optic disc area (*ATOH7*, *CDC7/TGFBR3*, and *SALL1*) were identified in early endophenotype studies of glaucoma.^{30,31} More recent GWAS have detected additional genetic factors that determine optic disc features.³² Similar studies also have identified many genetic factors associated with other endophenotypes of glaucoma, including the magnitude of IOP^{17,20} and central corneal thickness (CCT).^{21,33–35} There is some overlap between the genes identified by GWAS of endophenotypes and GWAS of glaucoma overall.

Elevated IOP in the absence of diagnostic features of glaucoma (optic nerve damage, visual field loss, or both) is classified as ocular hypertension (OHT).³⁶ The Ocular Hypertension Treatment Study (OHTS) was a multicenter treatment trial that investigated the efficacy of medical treatment of OHT to prevent development of POAG.³⁶ Of the 1636 individuals with OHT who were enrolled in the OHTS, POAG developed in 19% at 13 years of follow-up between 1994 and 2009.³⁷ Herein we report investigating the role of 10 previously reported glaucoma risk alleles and 3 alleles associated with optic nerve features in the participants of the OHTS. We tested OHTS participants at single nucleotide polymorphisms (SNPs) located in a total of 13 loci. Six of these loci were discovered with GWAS of POAG with high IOP (*ATOH7*, *CAV1/CAV2*, *CDKN2B-AS1*, *GAS7*, *SIX1/SIX6*, and *TMCO1*). We also tested SNPs in 5 loci that have been associated with glaucoma that occurs with low IOP (*CDKN2B-AS1*, chromosome 8q22, *ELOVL5*, *SRBD1*, and *TLR4*), one of which is also associated with glaucoma with high IOP. Finally, we evaluated SNPs at 6 loci that influence optic disc features (*ATOH7*, *CDC7/TGFBR3*, *CDKN2B-AS1*, *CHEK1*, *SALL1*, *SIX1/SIX6*), 3 of which are also associated with POAG. Since the onset of our study of these 13 factors, additional POAG risk factors have been detected, but were not included in our analysis.

Methods

Patient Cohort

Informed consent was obtained from all participants and approval for the study was granted by the institutional review boards of all participating institutions. A total of 1636 individuals with OHT were enrolled in the OHTS with inclusion and exclusion criteria that have been described previously.³⁶ Briefly, participants were required to have OHT defined as IOP of 24 mmHg or more, but 32 mmHg or less, in one eye, and IOP of 21 mmHg or more, but 32 mmHg or less, in the other eye at the time of enrollment after washout of any topical glaucoma medications. The OHTS participants were between 40 and 80 years of age inclusively and had normal and reliable baseline visual fields as determined by Humphrey 30-2 (Carl Zeiss Meditec, Dublin, CA) visual field testing and review by the OHTS visual field reading center. Finally, OHTS participants were judged to have normal optic nerve heads by both clinicians and review of stereoscopic optic disc photographs by the OHTS optic disc reading center. At the time of enrollment, all participants were judged to have OHT and none had been diagnosed with glaucoma. The demographics, including self-reported ethnicity, of the entire OHTS cohort have been described previously.³⁶

A subset (n = 1077) of the 1636 subjects of the OHTS were enrolled in an ancillary genetic study. Informed consent for genetic studies was obtained from all study participants. The DNA was obtained from peripheral blood samples using standard methods,³⁸ and high-quality DNA samples were obtained from 1063 of the 1077 participants (98.7%). The analysis sample investigated in the current study is described in Figure 1.

The OHTS participants were examined biannually for the development of POAG using a well-described set of criteria for optic nerve damage, visual field defects, or both.³⁶ By 2009, 209 of the 1063 participants (19.7%) who contributed DNA samples for genetic study had met study criteria for a diagnosis of glaucoma.

Genotyping

The cohort of 1063 OHTS participants that contributed DNA samples was studied as part of a GWAS to search for genes that determine quantitative features of glaucoma. Participants were genotyped at 1.1 million SNPs using Illumina Omni-1M-Quad microarrays (Illumina, San Diego, CA) in collaboration with the Center for Inherited Disease Research (www.cidr.jhmi.edu) and the National Human Genome Research Institute (www.genome.gov). An additional 24 masked duplicate samples and 50 control samples from the International HapMap Project also were genotyped to aid with quality control. All DNA samples were genotyped at the same time, on the same genotyping platform, and were plated in random order. Quality control and data cleaning of the genotypic data were conducted in collaboration with the Center for Inherited Disease Research. A total of 6 participants were excluded from this analysis because of unexpected duplicate enrollment (n = 2), ethnicity classification errors (n = 1), or unexpected relatedness (n = 3). The genotypes generated from the remaining 1057 samples all passed quality control thresholds with overall call rates of more than 87.9%. Individual SNPs that produced genotypes with call rates of less than 99% or that produced Hardy-Weinberg disequilibrium of less than 10⁻⁶ were eliminated from the study. Concordance of genotype calls between duplicate samples was 99.99% for masked OHTS duplicates and 99.7% for HapMap controls. Genotypes from 905 636 of the SNPs on the Omni-1M-Quad microarrays satisfied all quality control criteria for the 1057 study participants, were released by the Center for Inherited Disease Research, and have been posted at dbGaP on the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

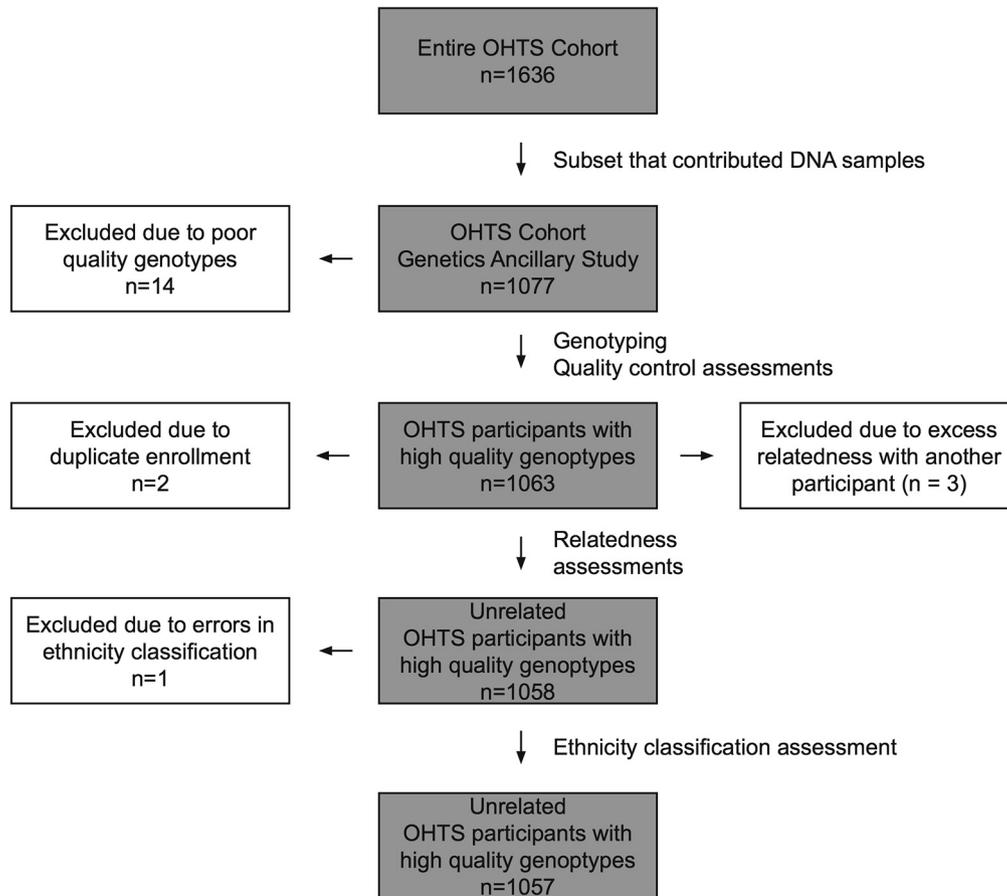


Figure 1. Flowchart showing the enrollment of Ocular Hypertension Treatment Study (OHTS) participants in a genetics ancillary study. Of the cohort of 1636 OHTS participants, 1077 contributed DNA samples and were part of a genome-wide association study. The genotypes of 1057 of these participants passed quality control metrics and were analyzed in this study.

Power Calculations

We calculated that our sample of 209 participants who were diagnosed with POAG (cases) and 848 participants who were not diagnosed with POAG has a more than 80% power to detect a 7.2% difference in risk factor allele frequency between cases and controls when the minor allele frequency is 10% among controls. The Bonferroni method was used to correct P values for multiple measures at an overall significance of 0.05.

Analysis of Genotypes

Genotypes of 1057 OHTS participants were analyzed further using GenomeStudio (Illumina) and PLINK version 1.07.³⁹ Multiple dimensional scaling was used as a covariate in linear regression to control for possible population stratification and was compared with self-reported ethnicity. Age and gender also were included as covariates.

Of 20 SNPs at 13 loci previously associated with POAG, 10 SNPs were present on the Omni1-Quad microarray and genotypes were readily available for analysis. For the remaining 10 SNPs, an alternate SNP was chosen using the HapMap linkage disequilibrium data (release 27). There were 2 cases in which a single SNP replaced a pair of SNPs in the *TLR4* gene. Using these alternates resulted in examining 20 unique SNPs at 13 previously associated loci (Table 2).

Statistical Analysis

The allele frequencies of 20 SNPs at 13 loci were compared between OHTS participants who demonstrated POAG ($n = 209$) and those who did not demonstrate POAG ($n = 848$) at 13 years of follow-up using a Mantel-Haenszel test to control for race. The allele frequencies of these SNPs in the 2 largest subpopulations (self-reported black persons, $n = 249$; non-Hispanic white persons, $n = 752$) also were compared separately using a chi-square test. A gene-based ($n = 13$) Bonferroni correction for multiple measures was used to set the threshold for significance at $P = 0.05/13 = 0.0038$.

The risk for glaucoma was assessed using the Kaplan-Meier survival analysis and Cox proportional hazards analysis. The proportion of OHTS participants that remained free of glaucoma at 6-month intervals was assessed for those initially randomized to treatment or observation. We calculated the Cox proportional hazard for POAG using the software package R (The R Project for Statistical Computing; <http://www.r-project.org>) with the same covariates as used previously to assess risk in the OHTS cohort. These covariates include: (1) age at enrollment, (2) IOP at enrollment (average between right and left eyes), (3) CCT (average between right and left eyes), (4) vertical cup-to-disc ratio at enrollment (average between right and left eyes), and (5) pattern standard deviation of Humphrey visual field testing at enrollment (average between right and left eyes). We added randomization to treatment or observation at enrollment, gender, and presence of *TMCO1* risk alleles to the Cox proportional hazard model.

Table 1. Demographics of the Ocular Hypertension Treatment Study Cohort and the Subset in the Genetics Study

| | Overall Ocular Hypertension Treatment Study Cohort (n = 1636) | Ocular Hypertension Treatment Study Participants in This Study (n = 1057) | P Value |
|--|---|---|---------|
| Mean age at enrollment (years) | 55.4 | 55.9 | 0.52 |
| Gender | | | 0.60 |
| Male | 705 (43.1%) | 461 (43.6%) | |
| Female | 931 (56.9%) | 596 (56.4%) | |
| Self-reported race | | | 0.51 |
| White | 1138 (69.6%) | 752 (71.1%) | |
| Black | 409 (25.0%) | 249 (23.6%) | |
| Hispanic | 59 (3.61%) | 36 (3.41%) | |
| Asian/Pacific Islander | 14 (0.856%) | 9 (0.851%) | |
| Other | 14 (0.856%) | 9 (0.851%) | |
| American Indian/Alaskan | 4 (2.44%) | 2 (0.189%) | |
| Cumulative proportion in whom POAG developed in the OHTS | | | 0.91 |
| Overall | 279 (17.1%) | 209 (19.8%) | |
| White | 170 (14.9%) | 124 (16.5%) | |
| Black | 91 (22.2%) | 70 (28.1%) | |
| Hispanic | 12 (20.3%) | 10 (27.8%) | |
| Asian/Pacific Islander | 2 (14.3%) | 2 (22.2%) | |
| Other | 3 (21.4%) | 2 (22.2%) | |
| American Indian/Alaskan | 1 (25.0%) | 1 (50.0%) | |

OHTS = Ocular Hypertension Treatment Study; POAG = primary open-angle glaucoma.

The distributions of gender, race, and ethnicity are closely matched between the entire Ocular Hypertension Treatment Study cohort (first column) and the subset of the cohort available for genetic study (second column).

Results

Study Participants

A total of 1636 participants were enrolled in the OHTS, and DNA samples were available from a subset of this cohort (n = 1077). These 1077 OHTS participants were genotyped at 1 051 295 SNPs as part of a quantitative traits study. After quality-control criteria were applied, 905 636 SNPs typed in 1063 participants were released for analysis. An additional 6 participants were removed from the study because of inadvertent duplicate enrollment in the OHTS (n = 2), excess relatedness (n = 3), or an ethnicity misclassification (n = 1). The remaining 1057 OHTS participants and their genotypes are the focus of this study (Fig 1).

The demographic information from 1057 OHTS subjects in the genetics study is shown in Table 1. The racial composition of this subset of the OHTS is 71.1% non-Hispanic white and 23.6% black and is similar to the entire OHTS cohort as described previously.³⁶ Over the course of 13 years of follow-up examinations, 209 of these 1057 OHTS participants (19.8%) met study criteria for a diagnosis of POAG, whereas 848 (80.2%) were not diagnosed with glaucoma.

Analysis of Previously Reported Genetic Factors for Primary Open-Angle Glaucoma

Prior studies reported associations between SNPs at 19 loci and POAG (including normal-tension glaucoma). Many additional loci associated with endophenotypes of glaucoma such as vertical cup-to-disc ratio also have been identified. We examined the role of SNPs at 13 loci that had been discovered at the time of our study's onset. Single nucleotide polymorphisms at 10 loci associated with POAG were investigated in our study (*ATOH7*,¹³ *CAVI/CAV2*,¹² *CDKN2B-AS1*,¹³⁻¹⁶ *CHR 8q22*,¹⁵ *ELOVL5*,²⁴ *GAS7*,¹⁷ *SIX1/*

SIX6,^{13,16} *SRBD1*,²⁴ *TLR4*,^{26,27} and *TMCO1*¹⁷). Of these 10 loci, *CDKN2B-AS1*, *CHR 8q22*, *ELOVL5*, *SRBD1*, and *TLR4* have been associated with normal-tension glaucoma. We also investigated 6 loci that have been associated with optic disc features (i.e., disc area or vertical cup-to-disc ratio): *ATOH7*, *CDC7/TGFBR3*,^{13,40} *CDKN2B-AS1*, *CHEK2*,⁴¹ *SALL1*,¹³ and *SIX1/SIX6* (3 of which are also associated with POAG). Genotype frequencies at each of these 13 loci were compared between 209 OHTS participants who were diagnosed with POAG and 848 OHTS participants who were not diagnosed with glaucoma using linear regression controlling for population stratification, gender, and age as covariates. When the entire OHTS cohort (n = 1057) was analyzed (Table 2), no SNPs at these 13 loci were associated with POAG with a threshold for significance corrected for multiple measures ($P < 0.0038$).

The OHTS cohort includes participants from several racial and ethnic groups (Table 1); however, the 2 largest subgroups are self-reported non-Hispanic whites (n = 752) and blacks (n = 249). When these groups were analyzed separately (Table 2), 1 locus (*TMCO1*) contained an SNP (rs4656461) with allele frequencies that were associated significantly with POAG among the non-Hispanic white subset of participants. The minor allele frequency of rs4656461 was 24.6% in non-Hispanic whites in whom POAG developed and 15.5% in those in whom POAG did not develop ($P = 0.00028$). Numerous clinical studies have established that age, IOP, and CCT are important risk factors for POAG. However, *TMCO1* genotypes were not associated with age, IOP, or CCT in the OHTS cohort ($P > 0.05$), suggesting that *TMCO1* is an independent risk factor for POAG. Moreover, the association between *TMCO1* and POAG in non-Hispanic white participants remained significant ($P = 0.000072$) when the data were reanalyzed using age, gender, IOP, and CCT as covariates, further indicating that *TMCO1* is associated independently with POAG in the OHTS cohort.

Table 2. Candidate Gene Association Study

| Locus/Gene | Original Single Nucleotide Polymorphism | Single Nucleotide Polymorphism Analyzed | Ocular Hypertension Treatment Study Cohort | | | Black Subgroup | | Non-Hispanic White Subgroup | | | |
|-------------------|---|---|--|--|-------------|--------------------------------------|--|-----------------------------|---------------------------------------|--|---------------|
| | | | Primary Open-Angle Glaucoma (n = 209) | No Primary Open-Angle Glaucoma (n = 848) | P Value | Primary Open-Angle Glaucoma (n = 70) | No Primary Open-Angle Glaucoma (n = 179) | P Value | Primary Open-Angle Glaucoma (n = 124) | No Primary Open-Angle Glaucoma (n = 628) | P Value |
| | | | MAF | MAF | | MAF | MAF | | | | |
| ATOH7 | rs1900004 | rs7916697 | 0.426 | 0.357 | 0.66 | 0.307 | 0.279 | 0.95 | 0.290 | 0.255 | 0.27 |
| CAV1/CAV2 | rs4236601 | rs2024211 | 0.318 | 0.325 | 0.94 | 0.336 | 0.344 | 0.76 | 0.315 | 0.326 | 0.77 |
| CDC7/TGFBR3 | rs1192415 | rs1192419 | 0.225 | 0.211 | 0.93 | 0.293 | 0.285 | 0.66 | 0.186 | 0.191 | 0.89 |
| CDKN2B-AS1 | rs1063192 | rs1063192 | 0.263 | 0.369 | 0.02 | 0.086 | 0.084 | 0.93 | 0.371 | 0.459 | 0.0060 |
| | rs2157719 | SNP9-22023366 | 0.263 | 0.364 | 0.03 | 0.086 | 0.078 | 0.77 | 0.371 | 0.452 | 0.012 |
| | rs4977756 | rs4977756 | 0.357 | 0.400 | 0.15 | 0.407 | 0.335 | 0.18 | 0.351 | 0.427 | 0.012 |
| CHEK2 | rs1547014 | rs1547014 | 0.361 | 0.346 | 0.34 | 0.479 | 0.489 | 0.42 | 0.298 | 0.315 | 0.42 |
| Chrom 8q22 | rs284489 | rs284489 | 0.431 | 0.396 | 0.39 | 0.393 | 0.444 | 0.24 | 0.339 | 0.348 | 0.66 |
| ELOVL5 | rs735860 | rs735860 | 0.278 | 0.355 | 0.06 | 0.093 | 0.098 | 0.69 | 0.387 | 0.431 | 0.093 |
| GAS7 | rs11656696 | rs11656696 | 0.323 | 0.348 | 0.79 | 0.236 | 0.249 | 0.69 | 0.367 | 0.377 | 0.85 |
| SALL1 | rs1362756 | rs1345467 | 0.270 | 0.278 | 0.95 | 0.314 | 0.355 | 0.76 | 0.246 | 0.256 | 0.95 |
| SIX1/SIX6 | rs10483727 | rs2057135 | 0.153 | 0.148 | 0.52 | 0.214 | 0.321 | 0.35 | 0.109 | 0.101 | 0.67 |
| SRBD1 | rs3213787 | rs17033801 | 0.022 | 0.027 | 0.90 | 0.000 | 0.008 | NA | 0.020 | 0.030 | 0.46 |
| TLR4 | rs12377632 | rs10759930 | 0.309 | 0.333 | 0.71 | 0.093 | 0.103 | 0.46 | 0.436 | 0.393 | 0.28 |
| | rs2149356 | rs1360094 | 0.452 | 0.423 | 0.41 | 0.257 | 0.254 | 0.68 | 0.290 | 0.336 | 0.20 |
| | rs10759930 | rs10759930 | 0.309 | 0.333 | 0.71 | 0.093 | 0.103 | 0.46 | 0.436 | 0.393 | 0.28 |
| | rs1927914 | rs1360094 | 0.452 | 0.423 | 0.41 | 0.257 | 0.254 | 0.68 | 0.290 | 0.336 | 0.20 |
| | rs1927911 | rs1927911 | 0.373 | 0.338 | 0.76 | 0.357 | 0.390 | 0.31 | 0.226 | 0.267 | 0.25 |
| | rs7037117 | rs1927906 | 0.220 | 0.160 | 0.41 | 0.414 | 0.430 | 0.45 | 0.093 | 0.084 | 0.11 |
| TMCO1 | rs4656461 | rs4656461 | 0.234 | 0.178 | 0.03 | 0.221 | 0.254 | 0.17 | 0.246 | 0.155 | 0.0028 |

MAF = minor allele frequency; NA = not applicable.

The threshold for a significant association corrected for multiple measures is $P = 0.0038$. The alleles of the single nucleotide polymorphism rs4656461 at the *TMCO1* locus are associated significantly with primary open-angle glaucoma in the non-Hispanic white subgroup.

Assessment of Risk for Primary Open-Angle Glaucoma Conferred by *TMCO1* Alleles at the rs4656461 Locus

At the onset of the OHTS, all participants were determined to be free of glaucoma damage. Over the course of the first 13 years of follow-up, 209 of 1057 participants who contributed DNA samples demonstrated incident cases of POAG. At 13 years, 59 of the 199 OHTS participants (29.6%) with *TMCO1* risk alleles still in the study had glaucoma, whereas 69 of the remaining 362 participants (19.1%) without *TMCO1* risk alleles had glaucoma. Overall, the proportion of participants with glaucoma at 13 years was 10.5% more in OHTS participants with *TMCO1* risk alleles than in those with no risk alleles.

The rate at which POAG was diagnosed was compared between OHTS participants with *TMCO1* risk alleles (either 1 or both alleles) and participants with no risk alleles using a modified Kaplan-Meier analysis (Fig 2A). Carriers of 1 or 2 *TMCO1* risk alleles were grouped together because of the relative rarity of participants with 2 risk alleles. Overall, participants with *TMCO1* risk alleles developed glaucoma at a higher rate than participants with no risk alleles ($P = 0.0128$). By 13 years, OHTS participants with *TMCO1* risk alleles had a 30.2% probability of glaucoma developing compared with a probability of 22.3% for those with no *TMCO1* risk alleles (Fig 2A). Thus, *TMCO1* risk alleles were associated with a 7.9% higher probability for glaucoma developing at 13 years.

Reduction of IOP is known to influence the risk for glaucoma developing. Consequently, we compared *TMCO1* risk allele frequencies between the subset of the OHTS participants that was randomized initially to receive IOP-lowering medications and the subset that was randomized initially to receive placebo (Table 3). *TMCO1* risk allele frequencies are the same in treated and untreated arms of the OHTS ($P = 0.35$). Moreover, the same

trend of increased rates of glaucoma among carriers of *TMCO1* risk alleles was observed when either initially treated or untreated subsets of the OHTS participants were investigated separately with Kaplan-Meier analysis (Supplemental Fig S1, available at www.aajournal.org).

The 2 largest racial subsets of the OHTS participants (non-Hispanic whites and blacks) also were studied separately with Kaplan-Meier analysis. Non-Hispanic whites who carried 1 or 2 *TMCO1* risk alleles demonstrated glaucoma at a statistically higher rate than those with no risk alleles (Fig 2B; $P = 0.0014$). These data showed that, after 13 years, non-Hispanic whites with high-risk *TMCO1* alleles had a 28.8% probability of having glaucoma compared with a probability of 17.1% for those with no *TMCO1* risk alleles (Fig 2B). These data suggested that *TMCO1* risk alleles were responsible for an 11.7% higher probability for glaucoma developing at 13 years. Further, Kaplan-Meier analysis showed that *TMCO1* risk alleles were associated with a higher risk of glaucoma developing in both the initially treated ($P = 0.00909$) or untreated ($P = 0.0304$) subgroups of non-Hispanic whites (Supplemental Fig S1, available at www.aajournal.org). No statistically significant difference in the rate of glaucoma was detected between the smaller cohorts of black OHTS participants with *TMCO1* risk alleles and black participants with no risk alleles.

Calculation of Risk

We analyzed the longitudinal OHTS data to quantify the POAG risk from *TMCO1* alleles using the Cox proportional hazards model. The original risk calculator for POAG in the OHTS cohort included (1) age, (2) IOP, (3) CCT, (4) vertical cup-to-disc ratio, and (5) pattern standard deviation of Humphrey visual field tests. We added initial randomization to treatment or observation, gender, and *TMCO1* genotype to our POAG risk calculator. We focused our analysis on the non-Hispanic white subset of the OHTS participants ($n = 752$)

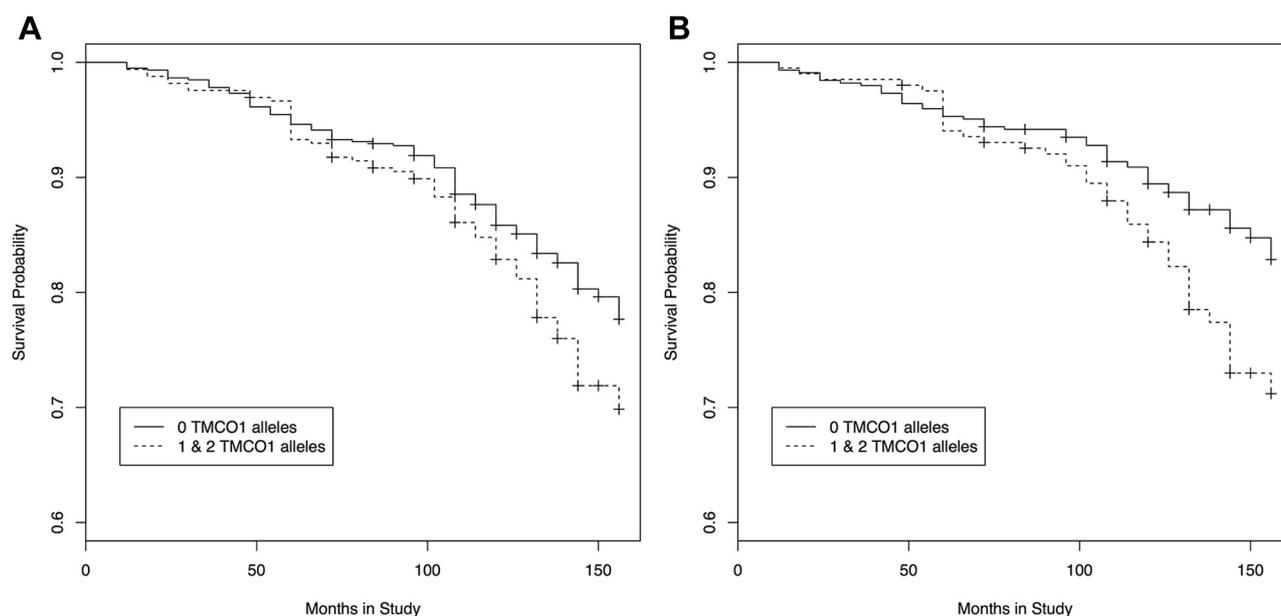


Figure 2. Kaplan-Meier analysis of Ocular Hypertension Treatment Study (OHTS) participants with *TMCO1* glaucoma risk factors. At the onset of the study, none of the OHTS participants had a diagnosis of glaucoma. At 6-month intervals, participants were evaluated for onset of glaucoma. Survival probability, which refers to the probability of remaining free of glaucoma, is plotted on the y-axis. Thus, the probability of glaucoma developing is equal to $1 -$ the probability of survival. Survival probability for participants with no *TMCO1* risk alleles is plotted with a solid line, whereas survival probability for participants with either 1 or 2 *TMCO1* risk alleles is plotted with a dashed line. **A**, Data plotted from all participants in the genetics ancillary study of the OHTS ($n = 1057$); **B**, Data plotted from the non-Hispanic white subset of the ancillary study ($n = 752$).

Table 3. Distribution of *TMCO1* Risk Alleles between Subsets of the Ocular Hypertension Treatment Study Cohort Randomized to Initial Observation or to Initial Treatment

| No. of <i>TMCO1</i> Risk Alleles (rs46546461) G Alleles | Entire Cohort | | | Non-Hispanic Whites | | | Blacks | | |
|---|------------------|-----------------------------|------------------------------|---------------------|-----------------------------|------------------------------|-----------------|-----------------------------|------------------------------|
| | Total (n = 1056) | Initially Treated (n = 546) | Initially Observed (n = 510) | Total (n = 751) | Initially Treated (n = 395) | Initially Observed (n = 346) | Total (n = 249) | Initially Treated (n = 126) | Initially Observed (n = 123) |
| 0 | 690 | 367 | 323 | 521 | 273 | 248 | 134 | 78 | 56 |
| 1 | 332 | 164 | 168 | 205 | 108 | 97 | 108 | 47 | 61 |
| 2 | 34 | 15 | 19 | 25 | 14 | 11 | 7 | 1 | 6 |
| | | P = 0.35 | | | P = 0.94 | | | P = 0.011 | |

TMCO1 risk alleles were present at the same frequency among Ocular Hypertension Treatment Study participants randomized to initial treatment or to initial observation when the entire cohort was analyzed (left columns). The *TMCO1* risk alleles also were present at the same frequency in white participants randomized to initial treatment or initial observation (middle columns), whereas there was a difference between groups among the black participants (right columns), which is likely because of the smaller sample size.

for which *TMCO1* alleles are associated significantly with POAG. In this model, *TMCO1* genotype was associated highly with risk for POAG (Table 4). *TMCO1* risk alleles had a hazard ratio of 1.73 per allele ($P = 0.00036$) and had an influence on risk for glaucoma that is on par with other clinical and demographic features of glaucoma (Table 4). A similar analysis with the black subset of the OHTS cohort did not produce hazard ratios that were statistically significant ($P = 0.085$).

Discussion

Observational and epidemiologic studies have provided strong evidence for genetic contributions to both the overall risk for a clinical diagnosis of glaucoma as well as many of the component quantitative features of disease, such as corneal thickness, IOP, and optic disc morphologic features.^{42–46} The first successes in genetic studies of glaucoma were achieved with investigations of Mendelian (single-gene) forms of POAG. Linkage studies of rare pedigrees identified mutations in genes (e.g., *MYOC*, *OPTN*, and *TBK1*) that are each capable of causing glaucoma with little influence from other genes and the environment.⁵ Risk factors also have been discovered for forms of glaucoma that have a complex basis involving many genes that contribute to pathogenesis, but are not causative on their own. For example, the *LOXLI* locus has been identified as a powerful risk factor for exfoliation syndrome (odds ratio, >20) and exfoliation glaucoma.⁴⁷ Several POAG risk factors also have been discovered, including *CAVI*/*CAV2*,

CDKN2B-AS1,^{11,13,15,48} *TMCO1*,¹¹ *ATOH7*,¹³ *SIX1/SIX6*,¹³ *GAS7*,¹⁷ *TLR4*,²⁶ *SRBD1*,²⁵ *ELOVL5*,²⁵ chromosome 8q22,¹⁵ *ABCA1*,¹⁸ *AFAP1*,¹⁸ *GMDS*,¹⁸ *PMM2*,¹⁹ *FNDC3B*,^{20,21} *TGFBR3*,²² *TXNRD2*,²³ *ATXN2*,²³ and *FOXCI*.²³ Each of these POAG risk factors was discovered with case-control association studies that provide estimates of their influence on the development of glaucoma (i.e., odds ratios). These factors each confer relatively modest risk for POAG, which suggests that numerous risk factors (genetic and environmental) must be present for disease to occur and that many more factors remain to be discovered.

The known glaucoma risk factors have been studied and validated further in patient cohorts from prior epidemiologic studies and treatment trials. Members of the Nurses' Health Study and the Health Professionals Follow-up Study were screened for glaucoma and included in a multicenter POAG study: Glaucoma Genes and Environment (GLAUGEN). The National Eye Institute Glaucoma Human Genetics Collaboration (NEIGHBOR) consortium has also conducted genetic studies of a large cohort of POAG patients collected from multiple centers in the United States.⁴⁹ Associations between *CDKN2B-AS1* and *SIX1/SIX6* were validated with these cohorts, and a new chromosome 8q22 risk factor locus was discovered.¹⁵ The Blue Mountains Eye Study (BMES) examined a cohort of 3654 predominantly white residents of a suburb of Sydney to determine the prevalence of glaucoma in this population (2.4% definite glaucoma).⁵⁰ Later DNA samples from BMES participants were used in

Table 4. Cox Proportional Hazards Analysis

| | Hazard Ratio | 95% Confidence Interval | P Value |
|--|--------------|-------------------------|------------|
| Age (decade) | 1.40 | 1.14–1.71 | 0.0011 |
| Gender (male) | 1.53 | 1.05–2.23 | 0.025 |
| IOP (per 2.7 mmHg [SE]) | 1.28 | 1.03–1.46 | 0.021 |
| CCT (per 37 μm [SE]) | 1.57 | 1.30–1.89 | 0.0000028 |
| Vertical cup-to-disc ratio (per 0.19 [SE]) | 1.62 | 1.33–1.96 | 0.00000096 |
| Visual Field defect (PSD, per 0.25 [SE]) | 1.20 | 0.99–1.45 | 0.070 |
| <i>TMCO1</i> (per risk allele) | 1.73 | 1.28–2.34 | 0.00036 |

CCT = central corneal thickness; IOP = intraocular pressure; PSD = pattern standard deviation; SE = standard error. The Ocular Hypertension Treatment Study risk calculator was adapted to include number of *TMCO1* risk alleles. Hazard ratios were normalized by SE.

the initial GWAS that discovered an association between risk factors *TMCO1* and *CDKN2B-AS1* and POAG.¹¹ Furthermore, a subset of the BMES participants were followed up over 10 years for the development of incident cases of POAG. In a recent report, a focused association study of BMES participants confirmed the association between several previously discovered risk factors and incident cases of glaucoma in the BMES cohort. Single nucleotide polymorphisms in *CDKN2B-AS1* (rs141829) and *SIX1/SIX6* (rs10483727) each were associated with increased risk for glaucoma developing with calculated odds ratios (univariate) of 1.67 and 1.66, respectively.⁵¹ A marginal association between *TMCO1* and incident glaucoma also was detected with an odds ratio of 1.74 (univariate), but did not survive multiple measure corrections.⁵¹

Our analysis of the OHTS cohort identified a significant association between *TMCO1* risk alleles and incident glaucoma cases among white participants. It is likely that the association was detected in whites and not in black participants primarily because of the larger sample size (Table 1). There is 9.1% difference in *TMCO1* minor allele frequency of the SNP rs4656461 between non-Hispanic whites with POAG and non-Hispanic whites with no POAG in the OHTS. If the same difference in minor allele frequency was observed in the smaller cohort of black OHTS participants, there would be only 36% power to detect a statistically significant difference at this locus. This low power suggests that small sample size may be one reason that an association was not detected between *TMCO1* and glaucoma in the black subset of the OHTS participants. It is also notable that the minor allele at the *TMCO1* locus (rs4656461) that is associated with glaucoma in non-Hispanic whites is more common among blacks without glaucoma (controls) than among blacks with glaucoma (Table 2). These data suggest that if there were an association between *TMCO1* and glaucoma in blacks that could be detectable with a larger cohort, the association likely would be in the opposite direction (i.e., with the major allele at rs4656461). Moreover, some of the other SNPs in our study, such as rs1063192 and SNP9-22023366 at the *CDKN2B-AS1* locus, have much lower minor allele frequencies in black participants than in non-Hispanic white participants, which also may have challenged our ability to detect statistically significant differences. These same *CDKN2B-AS1* SNP alleles that are less common in black subjects are “protective” and are associated with reduced risk for glaucoma. Lower frequency of these potentially beneficial alleles could contribute to the increased risk for glaucoma among blacks. Finally, other studies of African blacks have suggested that known glaucoma risk alleles may not be as strong in these populations and that a different set of risk factors may be more important.⁵² Our analysis of black OHTS participants is consistent with this conclusion.

Our failure to detect significant associations with additional known glaucoma risk factors most likely is the result of the relatively small size of the OHTS cohort. For example, nominal associations were detected at the *CDKN2B-AS1* locus ($P = 0.0060$) that did not survive multiple measures corrections for 13 loci. It is possible that

an association between *CDKN2B-AS1* or other factors might have been detectable if the OHTS cohort were larger or if less stringent multiple measures corrections were used.

Although *TMCO1* was associated first with POAG,¹¹ it was later identified as a gene that regulates the magnitude of IOP.^{17,53} Consequently, we considered that the association between *TMCO1* and glaucoma may be influenced by IOP. However, *TMCO1* remains associated with POAG in the OHTS cohort even when baseline IOP is controlled using linear regression. These results suggest that *TMCO1* may contribute risk for glaucoma that is independent of its influence on IOP. Because both the POAG cases and controls in the OHTS cohort were required to have high IOP through enrollment criteria, our analyses may be poorly suited to detecting IOP-dependent risk for POAG.

Since this analysis of 13 loci began, several additional SNPs associated with POAG have been identified with studies of larger patient cohorts: *ABAC1*, *AFAP1*, *GMD5*, *PMM2*, *FNDC3B*, *TGFBR3*, *TXNRD2*, *ATXN2*, and *FOXCI*. Future studies of these additional genes with the OHTS cohort may be warranted.

The primary goal of the OHTS randomized trial was to determine the safety and efficacy of IOP reduction achieved through medication on the risk for developing glaucoma. Rigorous longitudinal assessments for incident cases of glaucoma were conducted at 6-month intervals for more than 13 years and demonstrated that IOP reduction lowers risk for progression of OHT to POAG. We were able to use the same glaucoma incidence dataset to determine the influence of *TMCO1* risk alleles on glaucoma incidence. Analysis of non-Hispanic white OHTS participants demonstrated that those with 1 or 2 *TMCO1* risk alleles developed glaucoma at a significantly higher rate than those with no risk alleles ($P = 0.0014$; Fig 2B). After 13 years, 12% more of the non-Hispanic whites with *TMCO1* risk alleles demonstrated POAG than non-Hispanic whites without risk alleles. Half of the OHTS participants were randomized to initial observation and half to IOP reduction therapy. *TMCO1* alleles influence risk for glaucoma when initially observed or initially treated groups were analyzed separately (Supplemental Fig S1, available at www.aaojournal.org).

The OHTS cohort *TMCO1* genotype data also were examined with Cox proportional hazard models similar to those used in prior studies.⁵⁴ In addition to the clinical predictors used in the original model, our calculator included 3 more factors: (1) number of *TMCO1* risk alleles, (2) gender, and (3) initial randomization (observation or treatment). The number of *TMCO1* risk alleles is associated highly with development of glaucoma ($P = 0.00036$). In our model, each *TMCO1* risk allele had a hazard ratio of 1.73 and conferred more risk for glaucoma than the risk associated with a 0.1-larger baseline cup-to-disc ratio or with a 40- μ m thinner baseline CCT (Table 4).

Genetic analysis of the OHTS cohort provides evidence that *TMCO1* genotype is a strong predictor for the development of glaucoma. This analysis of *TMCO1* is based on individuals with OHT and may not be generalizable to all types of glaucoma suspects; however, its conclusions are

supported by the recent study of incident cases of POAG in the BMES by Burdon et al.⁵¹ Both Kaplan-Meier analyses and Cox proportion hazard models suggest that determining *TMC01* genotype may have clinical usefulness in assessing risk for glaucoma. The potential value of including *TMC01* genotypes in a glaucoma risk calculator is promising, but requires additional validation.

References

- Sommer A, Tielsch JM, Katz J, et al. Racial differences in the cause-specific prevalence of blindness in east Baltimore. *N Engl J Med*. 1991;325:1412-1417.
- Hyman L, Wu SY, Connell AM, et al. Prevalence and causes of visual impairment in The Barbados Eye Study. *Ophthalmology*. 2001;108:1751-1756.
- Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006;90:262-267.
- Kwon YH, Fingert JH, Kuehn MH, Alward WLM. Primary open-angle glaucoma. *N Engl J Med*. 2009;360:1113-1124.
- Fingert JH. Primary open-angle glaucoma genes. *Eye*. 2011;25:587-595.
- Stone EM, Fingert JH, Alward WLM, et al. Identification of a gene that causes primary open angle glaucoma. *Science*. 1997;275:668-670.
- Rezaie T, Child A, Hitchings R, et al. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science*. 2002;295:1077-1079.
- Fingert JH, Robin AL, Roos BR, et al. Copy number variations on chromosome 12q14 in patients with normal tension glaucoma. *Hum Mol Genet*. 2011;20:2482-2494.
- Alward WL, Fingert JH, Coote MA, et al. Clinical features associated with mutations in the chromosome 1 open-angle glaucoma gene (*GLC1A*). *N Engl J Med*. 1998;338:1022-1027.
- Thorleifsson G, Walters GB, Hewitt AW, et al. Common variants near *CAV1* and *CAV2* are associated with primary open-angle glaucoma. *Nat Genet*. 2010;42:906-909.
- Burdon KP, MacGregor S, Hewitt AW, et al. Genome-wide association study identifies susceptibility loci for open angle glaucoma at *TMC01* and *CDKN2B-AS1*. *Nat Genet*. 2011;43:574-578.
- Wiggs JL, Kang JH, Yaspan BL, et al. Common variants near *CAV1* and *CAV2* are associated with primary open-angle glaucoma in Caucasians from the USA. *Hum Mol Genet*. 2011;20:4707-4713.
- Ramdas WD, van Koolwijk LME, Lemij HG, et al. Common genetic variants associated with open-angle glaucoma. *Hum Mol Genet*. 2011;20:2464-2471.
- Nakano M, Ikeda Y, Tokuda Y, et al. Common variants in *CDKN2B-AS1* associated with optic-nerve vulnerability of glaucoma identified by genome-wide association studies in Japanese. *PLoS One*. 2012;7:e33389.
- Wiggs JL, Yaspan BL, Hauser MA, et al. Common variants at 9p21 and 8q22 are associated with increased susceptibility to optic nerve degeneration in glaucoma. *PLoS Genet*. 2012;8:e1002654.
- Osman W, Low S-K, Takahashi A, et al. A genome-wide association study in the Japanese population confirms 9p21 and 14q23 as susceptibility loci for primary open angle glaucoma. *Hum Mol Genet*. 2012;21:2836-2842.
- van Koolwijk LME, Ramdas WD, Ikram MK, et al. Common genetic determinants of intraocular pressure and primary open-angle glaucoma. *PLoS Genet*. 2012;8:e1002611.
- Gharahkhani P, Burdon KP, Fogarty R, et al. Common variants near *ABCA1*, *AFAP1* and *GMD5* confer risk of primary open-angle glaucoma. *Nat Genet*. 2014;46:1120-1125.
- Chen Y, Lin Y, Vithana EN, et al. Common variants near *ABCA1* and in *PMM2* are associated with primary open-angle glaucoma. *Nat Genet*. 2014;46:1115-1119.
- Hysi PG, Cheng C-Y, Springelkamp H, et al. Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma. *Nat Genet*. 2014;46:1126-1130.
- Lu Y, Vitart V, Burdon KP, et al. Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. *Nat Genet*. 2013;45:155-163.
- Li Z, Allingham RR, Nakano M, et al. A common variant near *TGFBR3* is associated with primary open angle glaucoma. *Hum Mol Genet*. 2015;24:3880-3892.
- Bailey JNC, Loomis SJ, Kang JH, et al. Genome-wide association analysis identifies *TXNRD2*, *ATXN2* and *FOXC1* as susceptibility loci for primary open-angle glaucoma. *Nat Genet*. 2016;48:189-194.
- Writing Committee for the Normal Tension Glaucoma Genetic Study Group of Japan Glaucoma Society, Meguro A, Inoko H, et al. Genome-wide association study of normal tension glaucoma: common variants in *SRBD1* and *ELOVL5* contribute to disease susceptibility. *Ophthalmology*. 2010;117:1331-1338.e5.
- Mabuchi F, Sakurada Y, Kashiwagi K, et al. Association between *SRBD1* and *ELOVL5* gene polymorphisms and primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2011;52:4626-4629.
- Shibuya E, Meguro A, Ota M, et al. Association of Toll-like receptor 4 gene polymorphisms with normal tension glaucoma. *Invest Ophthalmol Vis Sci*. 2008;49:4453-4457.
- Takano Y, Shi D, Shimizu A, et al. Association of Toll-like receptor 4 gene polymorphisms in Japanese subjects with primary open-angle, normal-tension, and exfoliation glaucoma. *Am J Ophthalmol*. 2012;154:825-832.e1.
- Suh W, Kim S, Ki C-S, Kee C. Toll-like receptor 4 gene polymorphisms do not associate with normal tension glaucoma in a Korean population. *Mol Vis*. 2011;17:2343-2348.
- Analysis of toll-like receptor rs4986790 polymorphism in Saudi patients with primary open angle glaucoma. *Ophthalmol Genet*. 2016:1-5.
- Ramdas WD, van Koolwijk LME, Ikram MK, et al. A genome-wide association study of optic disc parameters. *PLoS Genet*. 2010;6:e1000978.
- MacGregor S, Hewitt AW, Hysi PG, et al. Genome-wide association identifies *ATOX1* as a major gene determining human optic disc size. *Hum Mol Genet*. 2010;19:2716-2724.
- Springelkamp H, Mishra A, Hysi PG, et al. Meta-analysis of genome-wide association studies identifies novel loci associated with optic disc morphology. *Genet Epidemiol*. 2015;39:207-216.
- Lu Y, Dimasi DP, Hysi PG, et al. Common genetic variants near the brittle cornea syndrome locus *ZNF469* influence the blinding disease risk factor central corneal thickness. *PLoS Genet*. 2010;6:e1000947.
- Vitart V, Bencić G, Hayward C, et al. New loci associated with central cornea thickness include *COL5A1*, *AKAP13* and *AVGR8*. *Hum Mol Genet*. 2010;19:4304-4311.
- Vithana EN, Aung T, Khor CC, et al. Collagen-related genes influence the glaucoma risk factor, central corneal thickness. *Hum Mol Genet*. 2011;20:649-658.
- Gordon MO, Kass MA. The Ocular Hypertension Treatment Study: design and baseline description of the participants. *Arch Ophthalmol*. 1999;117:573-583.

37. Kass MA, Gordon MO, Gao F, et al. Delaying treatment of ocular hypertension: the ocular hypertension treatment study. *Arch Ophthalmol*. 2010;128:276-287.
38. Buffone GJ, Darlington GJ. Isolation of DNA from biological specimens without extraction with phenol. *Clin Chem*. 1985;31:164-165.
39. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559-575.
40. Axenovich T, Zorkoltseva I, Belonogova N, et al. Linkage and association analyses of glaucoma related traits in a large pedigree from a Dutch genetically isolated population. *J Med Genet*. 2011;48:802-809.
41. Mabuchi F, Sakurada Y, Kashiwagi K, et al. Association between genetic variants associated with vertical cup-to-disc ratio and phenotypic features of primary open-angle glaucoma. *Ophthalmology*. 2012;119:1819-1825.
42. Tielsch JM, Katz J, Sommer A, et al. Family history and risk of primary open angle glaucoma. The Baltimore Eye Survey. *Arch Ophthalmol*. 1994;112:69-73.
43. Becker B, Kolker AE, Roth FD. Glaucoma family study. *Am J Ophthalmol*. 1960;50:557-567.
44. Alsbirk PH. Corneal thickness. II. Environmental and genetic factors. *Acta Ophthalmol*. 1978;56:105-113.
45. Armaly MF. Genetic determination of cup/disc ratio of the optic nerve. *Arch Ophthalmol*. 1967;78:35-43.
46. Armaly MF. The genetic determination of ocular pressure in the normal eye. *Arch Ophthalmol*. 1967;78:187-192.
47. Thorleifsson G, Magnusson KP, Sulem P, et al. Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. *Science*. 2007;317:1397-1400.
48. Fan BJ, Wang DY, Pasquale LR, et al. Genetic variants associated with optic nerve vertical cup-to-disc ratio are risk factors for primary open angle glaucoma in a US Caucasian population. *Invest Ophthalmol Vis Sci*. 2011;52:1788-1792.
49. Wiggs JL, Hauser MA, Abdrabou W, et al. The NEIGHBOR Consortium Primary Open-Angle Glaucoma Genome-wide Association Study: Rationale, Study Design, and Clinical Variables. *J Glaucoma*. 2012;22:517-525.
50. Mitchell P, Smith W, Attebo K, Healey PR. Prevalence of open-angle glaucoma in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1996;103:1661-1669.
51. Burdon KP, Mitchell P, Lee A, et al. Association of open-angle glaucoma loci with incident glaucoma in the Blue Mountains Eye Study. *Am J Ophthalmol*. 2015;159:31-36.e1.
52. Liu Y, Hauser MA, Akafo SK, et al. Investigation of known genetic risk factors for primary open angle glaucoma in two populations of African ancestry. *Invest Ophthalmol Vis Sci*. 2013;54:6248-6254.
53. Ozel AB, Moroi SE, Reed DM, et al. Genome-wide association study and meta-analysis of intraocular pressure. *Hum Genet*. 2014;133:41-57.
54. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002;120:714-720. discussion 829-830.

Footnotes and Financial Disclosures

Originally received: March 29, 2016.

Final revision: August 19, 2016.

Accepted: August 23, 2016.

Available online: October 1, 2016. Manuscript no. 2016-632.

¹ Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, Iowa.

² Stephen A. Wynn Institute for Vision Research, University of Iowa, Iowa City, Iowa.

³ Department of Biostatistics, College of Public Health, University of Iowa, Iowa City, Iowa.

⁴ Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri.

Financial Disclosure(s):

The author(s) have made the following disclosure(s): T.E.S.: Equity owner – Bio::Neos (Coralville, IA)

J.H.F.: Unrelated Research Funding – Regeneron

Supported in part by the National Institutes of Health, Bethesda, Maryland (grant nos.: R01 EY018825 [J.H.F.], X01 HG005248 [J.H.F.], R01 EY023512 [J.H.F.], U10 EY009307 [M.A.K.], UG1 EY025180 [M.A.K.], U10 EY009341 [M.O.G.], and UG1 EY025182 [M.O.G.]).

Author Contributions:

Conception and design: Scheetz, Gordon, Kass, Wang, Fingert

Analysis and interpretation: Scheetz, Faga, Ortega, Roos, Gordon, Kass, Wang, Fingert

Data collection: Scheetz, Faga, Ortega, Roos, Gordon, Kass, Wang, Fingert

Obtained funding: none

Overall responsibility: Scheetz, Faga, Ortega, Roos, Gordon, Kass, Wang, Fingert

Abbreviations and Acronyms:

BMES = Blue Mountains Eye Study; **CCT** = central corneal thickness; **GWAS** = genome-wide association studies; **IOP** = intraocular pressure; **MAF** = minor allele frequency; **OHT** = ocular hypertension; **OHTS** = Ocular Hypertension Treatment Study; **POAG** = primary open-angle glaucoma; **SNP** = single nucleotide polymorphism; **TMC01** = transmembrane and coiled-coil domains 1.

Correspondence:

John H. Fingert, MD, PhD, Department of Ophthalmology and Visual Sciences, College of Medicine, University of Iowa, 3111B Carver Biomedical Research Building, 375 Newton Road, Iowa City, IA 52242. E-mail: john-fingert@uiowa.edu.